

Attorney Docket No.: P-514 (TI-0011)
Inventors: Paul D. Taylor
Serial No.: 09/756,070
Filing Date: January 16, 2001
Page 2

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (previously presented): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

- (a) applying the mixture to an anion-exchange solid;
- (b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes.

Claim 2 (currently amended): A method of claim 1 wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9, said mobile phase comprising:

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 3

an eluting salt composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, or mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate or mixtures thereof;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5~~7~~_i

an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M~~7~~_i.

Claim 3 (original): A method of claim 2 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

Claim 4 (currently amended): A method of claim 2 wherein said cation is selected from the group consisting of dialkylammonium,

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 4

trialkylammonium and tetraalkylammonium, wherein the alkyl groups consist of any combination of methyl and ethyl.

Claim 5(currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

- (a) applying the mixture to an anion-exchange solid;
- (b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, and contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 said mobile phase comprising an eluting salt composed of equal concentrations of a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium, or mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl and an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate or mixtures thereof; ~~(c)~~ a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and ~~(d)~~ an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5 M to approximately 2.0 M, and

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 5

wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes and wherein said cation comprises choline.

Claim 6 (original): A method of claim 2 wherein said cation comprises guanidinium.

Claim 7 (original): A method of claim 2 where said cation comprises sodium.

Claim 8 (original): A method of claim 2 wherein said anion is formate or chloride.

Claim 9 (original): A method of claim 2 wherein said mobile phase includes a metal chelating agent.

Claim 10 (currently amended): A method of claim 9 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 6

nitroso- β -naphthol, nitroso-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, $\alpha, \alpha', \alpha''$ -terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium ~~diethyldithiocarbarbamate~~ diethyldithiocarbarbamate, zinc, dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

Claim 11 (original): A method of claim 1, wherein said solid is comprised of a silica, polysaccharide or synthetic polyolefin backbone.

Claim 12 (original): A method of claim 11 wherein said polyolefin is a polystyrene or polyacrylic.

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 7

Claim 13 (original): A method of claim 1, wherein said solid comprises a polyacrylic backbone.

Claim 14 (original): A method of claim 1, wherein said solid comprises diethylaminoethyl functional groups.

Claim 15 (original): A method of claim 1, wherein said solid comprises polyethyleneimine functional groups.

Claim 16 (original): A method of claim 1, wherein said solid comprises particles with an average diameter between approximately 2 micron and 10 micron.

Claim 17 (original): A method of claim 1, wherein the solid is substantially nonporous.

Claim 18 (original): A method of claim 1, wherein said solid comprises a polystyrene backbone.

Claim 19 (original): A method of claim 1, wherein said mobile phase contains an organic solvent selected from the group

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 8

consisting of methanol, ethanol, acetonitrile, ethyl acetate, formamide, 2-propanol, and N-methyl pyrrolidone.

Claim 20 (original): A method of claim 1 wherein said mobile phase contains less than about 40% by volume of said organic solvent.

Claim 21 (original): A method of claim 1 wherein said eluting is carried out at a column temperature greater than about 50°C.

Claim 22 (original): A method of claim 1 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

Claim 23 (original): A method of claim 1 wherein the concentration of said eluting salt is continuously increased.

Claim 24 (original): A method of claim 1 including analyzing the mobile phase after the elution step (b) for the concentration of said DNA molecules.

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 9

Claim 25 (currently amended): A method of claim 24 wherein the concentration of said DNA molecules is measured by ultraviolet absorbance in the approximate wavelength range of about 250_nm to about 290_nm.

Claim 26 (original): A method of claim 1 wherein the total time required to complete said method is between about 2 minutes and about 30 minutes.

Claim 27 (original): A method of claim 1 wherein the concentration of organic solvent is systematically increased.

Claim 28 (previously presented): A method of claim 1 where said solid is contained in a column of cylindrical geometry.

Claim 29 (previously presented): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

- (a) applying the mixture to an anion-exchange solid,
- (b) eluting the solid of step (a) with a mobile phase containing an eluting salt and a buffer, where said eluting is carried out under conditions effective to at least partially

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 10

denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from said homoduplexes.

Claim 30 (currently amended): A method of claim 29, wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl;

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claims 31-32 (canceled).

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 11

Claim 33 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture comprising:

(a) applying the mixture to an anion-exchange solid, and

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from said homoduplexes and further solid of step(a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of a cation selected from the group consisting of dialkylammonium, trialkylammonium, and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl;

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; wherein the concentration of eluting salt is systematically increased from approximately 0.5 M to approximately 2.0 M and wherein said cation comprises choline.

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 12

Claim 34 (currently amended): A method of claim 30 wherein said cation comprises sodium.

Claim 35 (original): A method of claim 30 wherein said mobile phase includes a metal chelating agent.

Claim 36 (currently amended): A method of claim 35 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitros-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, $\alpha, \alpha', \alpha''$ -terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol,

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 13

mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium
~~diethyldithiocarbamate~~ diethyldithiocarbamate, zinc
dibenzoyldithiocarbamate, deferoxamine mesylate, crown ethers, and
mixtures of any one or more of the above.

Claim 37 (original): A method of claim 30 wherein said cation
comprises guanidinium.

Claim 38 (original): A method of claim 30 wherein said anion
is formate or chloride.

Claim 39 (original): A method of claim 30 wherein the
eluting salt is systematically increased from approximately 1.0M to
approximately 2.0M.

Claim 40 (original): A method of claim 30 including analyzing
the mobile phase eluting from the column for the presence of DNA.

Claim 41 (original): A method of claim 30 wherein said
eluting is carried out at a column temperature greater than about
50°C.

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 14

Claim 42 (original): A method of claim 30 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

Claims 43-63 (canceled).

Claim 64 (previously presented): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising of equal concentrations of:

a cation;

an anion;

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 15

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claim 65 (canceled).

Claim 66 (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said ~~heteroduplexes~~ heteroduplexes from said homoduplexes;

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 16

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising: an eluting salt comprising:

betaine at a concentration in the range of about 0.5M to about 6M;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and,

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claim 67 (original): A method of claim 66 wherein the eluting is carried out at a column temperature greater than about 50°C.

Claim (currently amended): A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

(a) applying the mixture to an anion-exchange solid;

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, where

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 17

said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising equal concentrations of:-

a cation;

an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and wherein the eluting is carried out at a column temperature greater than about 50°C,

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

Claims 69-71 (canceled).

Claim 72 (original): The method of claim 1, where prior to said applying step the DNA molecules are amplified using the polymerase chain reaction and the amplified DNA molecules are

Attorney Docket No.: **P-514 (TI-0011)**
Inventors: **Paul D. Taylor**
Serial No.: **09/756,070**
Filing Date: **January 16, 2001**
Page 18

denatured and renatured to form a mixture of heteroduplex and homoduplex DNA molecules.